



Conference Abstract

Diatom metabarcoding reveals differences in ecological preferences among genetic variants within some key species for biomonitoring

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Abstract

We applied DNA metabarcoding to evaluate the ecology of genetic variants within several diatom species that are important for biomonitoring. Benthic diatoms are widely used as bioindicators for biomonitoring programmes, including those for European rivers demanded by Water Framework Directive (WFD). Morphological identification of diatoms at species level is required for assessing the ecological status in biomonitoring programmes. However, this is a time-consuming task and requires expert knowledge. In addition, closely related species, which sometimes are scarcely distinguishable on the basis of their morphology, can show different ecological preferences; these may even vary within a single diatom species. Not being able to identify the different ecological preferences shown by the genetic variants of a single species or closely related species, might have consequences for biomonitoring programmes, especially if such differences occur within common species.

The key diatom species that we studied were: *Fistulifera saprophila* (FSAP), widely regarded as a marker for elevated nutrient levels, organic pollution and hence poor ecological status; *Achnanthidium minutissimum* (ADMI), which usually indicates good

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ecological status; and *Nitzschia inconspicua* (NINC) and *N. soratensis* (NSTS), two species that are widely separated phylogenetically but almost impossible to distinguish in the light microscope. Our dataset was based on high-throughput sequencing using a 312-bp *rbcL* marker. We used the denoising pipeline DADA2 to infer amplicon sequence variants (ASVs) from 554 environmental samples from river biomonitoring campaigns in Catalonia (NE Spain) and France. Ecological groupings of ASVs were distinguished according to their environmental responses given by Threshold Indicator Taxa ANalysis (TITAN); the environmental parameters that most influenced the occurrence of these groupings were tested using boosted regression trees.

We could distinguish three different ecological groupings of ASVs within ADMI and three within FSAP. In each species two of the groupings were clearly separated by their opposite responses to calcium and conductivity and boosted regression trees showed that for three out of four of these groupings, these two variables were among the most important variables for explaining the ASV distributions. The third grouping in FSAP had a negative response to total organic carbon and a positive response to altitude and hence was better represented in less organically polluted waters and higher ecological status than is generally assumed for FSAP.

Our analyses did not identify ecological groupings of ASVs within NINC and NSTS but confirmed earlier studies, based on more limited sampling, that indicated different preferences of these species. Conductivity and calcium were the variables that most influenced the occurrence of NINC and NSTS, NINC being better distributed in waters with higher levels of calcium and conductivity than NSTS.

Our findings indicate the potential use of DNA metabarcoding for distinguishing the ecological preferences of genetic variants within a single species or closely related species. This information, coupled with the broad knowledge generated over many years using traditional microscope-based identifications, will facilitate the development of more accurate biological indexes for the biomonitoring programmes of the future.

Keywords

ASV, Benthic diatoms, Water Framework Directive, Environmental DNA, *rbc*L, Ecological preferences, Genetic variation, Rivers

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